Sap flow of three co-occurring Mediterranean woody species under varying atmospheric and soil water conditions

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Summary We studied the seasonal patterns of water use in three woody species co-occurring in a holm oak forest in northeastern Spain. The three species studied, Quercus ilex L., Phillyrea latifolia L. and Arbutus unedo L., constitute more than 99% of the total basal area of the forest. The study period included the dry seasons of 1999 and 2000. Water use was estimated with Granier-type sap flux sensors. Standard meteorological variables, soil water content and leaf water potentials were also monitored. All monitored individuals reduced leaf-related sap flow (Ql) during the summer, concurrent with an increase in soil moisture deficit (SMD). Despite similar maximum Ql between species, the decline in Ql with increasing SMD was species-dependent. The average reduction in Ql between early summer and the peak of the drought was 74% for A. unedo (n = 3), 58% for P. latifolia (n = 3) and 87% for Q. ilex (n = 1). The relationship between canopy stomatal conductance (Gs) and vapor pressure deficit (D) changed during the course of the drought, with progressively lower Gs for any given D. Summertime reductions of Ql and Gs were associated with between-species differences in vulnerability to xylem embolism, and with the corresponding degree of native embolism (lowest in P. latifolia and highest in Q. ilex). Our results, combined with previous studies in the same area, outlined differences among the species studied in manner of responding to water shortage, with P. latifolia able to maintain water transport at much lower water potentials than the other two species. In an accompanying experiment, A. unedo responded to an experimental reduction in water availability by reducing Ql during the summer. This species also modified its water use between years according to the different seasonal patterns of precipitation. These results are discussed in relation to the possible impacts that climate change will have on Q. ilex-dominated forests.

Keywords: Arbutus unedo, drought, Phillyrea latifolia, Quercus ilex, sap flux, water use.

Introduction The climate in the Mediterranean basin is characterized by an acute summer drought that, according to most climatic scenarios, will intensify as a result of climate change (IPCC 2001). In this context, a proper understanding of the effects of water shortage is required if predictions of the impact of climate change on Mediterranean vegetation are to be made (Borghini et al. 1998). The long-term measurement of water use of co-occurring species in relation to environmental variables is a powerful approach for studying the response of plants to water availability, particularly when the species studied show contrasting adaptations to drought (e.g., Pataki et al. 2000). Because plant responses are likely to be influenced by different environmental variables depending on the temporal scale considered (Pataki et al. 1998), it is important that data are gathered at a sufficiently high temporal resolution. This requirement is fulfilled by sap flow studies because they provide estimates of transpiration with a temporal resolution of less than one day (Granier et al. 1996).

Evergreen forests dominated by holm oak (Quercus ilex L.) are one of the most important vegetation types in the Mediterranean basin. In Spain, for example, holm oak forests constitute 25% of the total forested area (Terradas 1999). Arbutus unedo L. and Phillyrea latifolia L. co-occur in holm oak forests throughout the Mediterranean, typically forming a lower tree layer under the Q. ilex canopy. Although the three species are evergreen and have similar distributions (Bolòs and Vigo 1990–1995), previous studies have shown that they differ markedly in water relations and resistance to drought (Table 1). In contrast to the other two species, P. latifolia exhibits little stomatal control over water loss and maintains physiological activity even when leaf water potentials are low (Tretiach 1993, Castell et al. 1994, de Lillis and Mirkone 1994, Peñuelas et al. 1998). Within a site, P. latifolia tends to occupy the driest areas, whereas A. unedo is usually restricted to the most humid microenvironments. However, recent
droughts in eastern Spain, particularly in summer 1994, have shown that *Q. ilex* is closer to its limit in coping with water stress than the other two species. In some areas, 80% of *Q. ilex* individuals lost all their foliage as a result of the 1994 drought (Lloret and Siscart 1995). Most of these trees resprouted, although mortality was about 15% in many populations (Peñuelas et al. 1998, 2000). Therefore, it has been hypothesized that *Q. ilex* may be substituted for *P. latifolia* in the most water-limited areas of the Mediterranean if the climate becomes drier (Peñuelas et al. 1998, 2000, Martínez-Vilalta et al. 2002a).

A recent study (Martínez-Vilalta et al. 2002b) has shown that *A. unedo*, *P. latifolia* and *Q. ilex* have contrasting hydraulic properties, which partially explain the observed differences in water relations and drought resistance. In particular, *P. latifolia* is much more resistant to xylem embolism than the other species, whereas *A. unedo* is slightly more resistant than *Q. ilex* (Table 1). In the present study, we used daily sap flow measurements to evaluate seasonal patterns of water use in these species, with the aim of integrating previous knowledge on their water relations and understanding the underlying mechanisms that explain their contrasting responses to seasonal drought. It was hypothesized that: (1) consistent with the previous considerations, the reduction of sap flow during a summer drought would follow a pattern of *Q. ilex* > *A. unedo* > *P. latifolia*; (2) the relationship between canopy stomatal conductance and vapor pressure deficit (*D*) would change accordingly during the drought period, with *Q. ilex* and *A. unedo* showing the largest increase in stomatal sensitivity to *D*; and (3) because water is a limiting resource in the community studied, its use would respond to differences in seasonal water availability between years and to an experimental reduction of water availability.

### Materials and methods

#### Study site

The site was located in a forested area in the Prades Mountains, NE Spain (41°13′ N, 0°55′ E; 990 m a.s.l.). The climate is Mediterranean, with a mean annual rainfall of 537 mm (1981–1995) and moderate temperatures (10.0 °C mean at Prades, 1000 m a.s.l.). Additional information about the study area can be found in Hereter and Sánchez (1999). Experimental plots were located on the south-facing upper slopes of the Torners valley (about 35% slope). The substrate is fractured schist, and soils are xerochrepts with a clay-loam texture. Soil depth is 65 ± 3 cm. The forest is dominated by *Q. ilex*, *P. latifolia* and *A. unedo*, which constitute ≥ 99% of the total basal area in the two study plots (Table 2). Canopy height is 4–7 m and LAI is = 4.5 (Sala et al. 1994). This community is known to be limited by water availability (Rodà et al. 1999). The study period included the dry seasons of years 1999 and 2000 (Figure 1).

#### Experimental design

This study was part of a drought simulation experiment, which included eight 15 × 10 m plots (four control and four drought-exposed), located at the same altitude along the slope. However, sap flow sensors were installed in only two of the plots (one assigned to each treatment), and we focused only on those in this study. The distance between the edges of these two plots was about 7 m. In the drought treatment, water availability to trees was restricted from March 1999 until the end of the study period by plastic sheets and collectors suspended 50–100 cm above the soil, and 1-m deep trenches on the upper sides of the plots to exclude lateral water flow. The plastic structures were made with transparent PVC and covered 30% of the surface area of the plots. The effectiveness of plastic sheets in excluding throughfall was tested in the drought-exposed plot by monitoring soil water content with three groups of paired time-domain-reflectometry (TDR) sensors over the entire study period. Within each group, one sensor was placed under a plastic sheet and the other in an adjacent, uncovered position. Water content was, on average, 3% units lower under the sheets than in the uncovered positions. Although the effect was not significant when integrated over the study period (*P* = 0.15, two-way ANOVA with position and sampling date as repeated measurement factors), it became significant after the rain events (*P* < 0.05, paired *t*-tests).

### Table 1. Mean (± 1 SE) xylem hydraulic and water-use-related parameters for *Arbutus unedo*, *Phillyrea latifolia* and *Quercus ilex* at the study site. Different letters indicate significant differences between species.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Tissue</th>
<th>Arbutus unedo</th>
<th>Phillyrea latifolia</th>
<th>Quercus ilex</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf:sapwood area ratio (× 10⁻² m² m⁻³)¹</td>
<td>Root</td>
<td>9.5 ± 0.5 a</td>
<td>8.8 ± 0.3 a</td>
<td>24.2 ± 4.2 b</td>
</tr>
<tr>
<td>Carbon isotope discrimination (δ¹³C; %)²</td>
<td>Stem</td>
<td>–25.0 ± 0.3 a</td>
<td>–27.0 ± 0.4 b</td>
<td>–25.9 ± 0.3 b</td>
</tr>
<tr>
<td>Specific conductivity (× 10⁻¹ m² MPa⁻¹ s⁻¹)³</td>
<td>Root</td>
<td>15.9 ± 5.1 a</td>
<td>18.9 ± 8.9 a</td>
<td>60.1 ± 37.0 a</td>
</tr>
<tr>
<td></td>
<td>Stem</td>
<td>5.4 ± 1.1 a</td>
<td>4.1 ± 0.8 a</td>
<td>25.0 ± 2.2 b</td>
</tr>
<tr>
<td>Leaf-specific conductivity (× 10⁻⁷ m² MPa⁻¹ s⁻¹)³</td>
<td>Root</td>
<td>2.3 ± 0.5 a</td>
<td>2.2 ± 0.5 a</td>
<td>12.0 ± 1.7 b</td>
</tr>
<tr>
<td>Xylem pressure at 50% embolism (MPa)³</td>
<td>Stem</td>
<td>–1.2 ± 0.2 a</td>
<td>–5.3 ± 0.9 b</td>
<td>–1.2 ± 0.5 a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>–3.1 ± 0.5 a</td>
<td>–6.6 ± 1.3 b</td>
<td>–2.0 ± 0.4 a</td>
</tr>
</tbody>
</table>

¹ Data from this study and from Ogaya et al. (2003).
² M. Mangiron (unpublished results).
³ Martínez-Vilalta et al. (2002b).
The composition of the plant community was similar at the two plots studied (Table 2). Photosynthetically active radiation (PAR) intercepted by the canopy was measured with a ceptometer (Decagon SunFleck Ceptometer, Pullman, WA) in each plot. The results indicated no between-plots difference in either initial leaf area index (LAI) or in LAI dynamics during the study (data not shown). Seasonal variation in intercepted PAR was low, with a maximum variation of 15% measured over the study period in one of the plots and 10% in the other, suggesting that LAI was approximately constant.

Atmospheric and soil moisture measurements

Sensors for measuring temperature and relative humidity (Model 50Y, Campbell Scientific, Logan, UT), solar radiation (Campbell SP1110 pyranometer) and wind speed (Campbell A100R switching anemometer) were located on a mast at approximately crown height. The mast and a standard rain gauge (Campbell SP1110 pyranometer) and wind speed (Campbell ARG100 tipping bucket rain gauge; 1.5 m height) were counted.

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Leaf water potential

Leaf water potentials were measured seasonally throughout the study period with a pressure chamber (PMS Instruments, Corvallis, OR) (Scholander et al. 1965). On each sampling date, shoot tips from the trees monitored with sap flow sensors or adjacent individuals ($n \geq 3$ individuals per species, one shoot tip per individual) were measured at predawn (just before sunrise) (0300–0500 h, solar time) and at midday (1100–1300 h). The time lag between shoot excision and measurement was always < 1 min. Shoot tips were not bagged before measurement. Arbutus unedo individuals were measured only during the year 2000.

Sap flow

Stem flux was monitored with 20-mm-long, constant heat flow gauges constructed according to Granier (Granier 1985, 1987). Within each of the two study plots, sap flow of three dominant trees per species was measured (Table 3). Sensors were installed between November 1998 and February 1999, except those on A. unedo individuals in the drought-exposed plot, which were installed in October 1999. A probe pair was inserted radially into the stem of each tree at breast height after removing the bark to expose the outer surface of the sapwood. The vertical separation between probes was approximately 15 cm. The temperature difference between the probes was recorded continuously to obtain sap flux density by means of the equation derived empirically by Granier (1985, 1987). Daily maximum temperature differences were determined from stored data and used to estimate temperature differences under zero flow conditions. This variable was nearly constant during the study (the slope of the relationship between the daily maximum electromotive force produced by the thermocouples and time was between $-1 \times 10^{-3}$ and $+1 \times 10^{-3}$ mV day$^{-1}$; the coefficient of variation of maximum electromotive force was $3.38 \pm 0.49\%$), suggesting that no substantial variation in the thermal properties of the surrounding wood occurred during the measurements. Sensors and trunks were insulated with glass wool to minimize ambient temperature gradients, and sensors were oriented to the north to avoid azimuthal effects.
Sap flow data were sampled at 5-s intervals and recorded by a data logger. The thickness of active sapwood was determined with an increment corer at the end of the study. The depth of active sapwood was estimated from the translucency of the wood in *A. unedo* and *P. latifolia*, and by stereoscopic examination in *Q. ilex*. Sapwood depth ranged between 18.7 and 35.8 mm (Table 3). In the only case where sapwood depth was less than the 20-mm sensor length, the temperature difference was corrected according to Clearwater et al. (1999). Because the species studied are diffuse-porous, when sapwood depth exceeded sensor length, sap flux density was assumed to be uniform over the entire sapwood depth. However, this assumption is not critical because we were interested mainly in the comparison of sap flow among species and, in most cases, in the comparison of values from the same trees at different periods. The area of active sapwood was obtained by averaging the thickness of sapwood at both ends of the core. The allometric relationships obtained in the same study area by Ogaya et al. (2003) for *P. latifolia* and *Q. ilex*, and by Lledó (1990) for *A. unedo*, were used to calculate leaf-related sap flow ($Q_l$, kg m$^{-2}$ day$^{-1}$). Diurnal values of $Q_l$ were summed to daily values to avoid the complications introduced by stem capacitance (Oren and Pataki 2001). Nomenclature follows that of Edwards et al. (1996).

**Whole-plant hydraulic conductance and canopy stomatal conductance**

When data were available, measurements of leaf water potentials were combined with $Q_l$ to calculate whole-plant hydraulic conductance ($k_{S-L}$; kg m$^{-2}$ MPa$^{-1}$ s$^{-1}$; Wullschleger et al. 1998):

$$k_{S-L} = \frac{Q_{l,max}}{(\Psi_{PD} - \Psi_{MD})}$$

where $\Psi_{PD}$ is predawn water potential, $\Psi_{MD}$ is midday water potential and $Q_{l,max}$ is leaf-related sap flow during peak transpiration at midday.

Canopy stomatal conductance ($G_s$; mm s$^{-1}$) can be derived from sap flow measurements, based on the assumption that sap flux density scaled by sapwood-to-leaf area ratio (i.e., $Q_l$) is equal to transpiration per unit of leaf area. For species with small leaves, with strong aerodynamic coupling to the atmosphere, the Penman-Monteith equation can be simplified, leading to the following relationship (Whitehead and Jarvis 1981, Pataki et al. 1998):

$$G_s = \frac{\gamma \lambda Q_l}{\rho c_p D}$$

where $\gamma$ is the psychrometric constant (kPa K$^{-1}$), $\lambda$ is the latent heat of vaporization of water (J kg$^{-1}$), $\rho$ is the density of air (kg m$^{-3}$) and $c_p$ is the specific heat of air at constant pressure (J kg$^{-1}$ K$^{-1}$). We used daily means of all quantities, excluding values of $D < 0.1$ kPa (Phillips and Oren 1998). In our case, the assumption of strong coupling to the atmosphere was tested by comparing $G_s$ calculated from Equation 2 with boundary layer conductance ($g_{st}$), estimated from daily mean wind speed ($u$; m s$^{-1}$) using $g_{st} = 6.62(u/\lambda)^{0.5}$ (Jones 1992). Based on the maximum leaf dimension ($d$) of 0.01 m, the ratio $g_{st}/G_s$ was $> 10$ for more than 95% of days in all studied trees, supporting the conclusion that calculated $G_s$ was dominated by stomatal aperture rather than by the boundary layer (Whitehead and Jarvis 1981). Leaf size and shape were similar in the species

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**Figure 1.** Daily values of maximum and minimum air temperature ($T_{air}$), cumulative water balance ($R = \text{rainfall}; E_T = \text{potential evapotranspiration}$; and SMD = soil moisture deficit) and volumetric soil water content ($\theta$) at two different depths during 1999 and 2000 at the study site. Shaded areas indicate the periods in which sap flow was monitored. Bars for $\theta$ represent standard errors.
studied. In applying Equation 2, we assumed that there was no vertical gradient of $D$ throughout the canopy.

**Data analysis**

Soil water content, leaf water potentials and $k_{s,4}$ were compared among treatments or among species using repeated measurements ANOVA. Because we were interested in the drought response of the studied species, our analysis of sap flow was restricted to measurements conducted from late spring (period with maximum flow) to late summer/early autumn (period with the lowest values). Because of sensor malfunction, the analysis of sap flow was limited to control plots in 1999 ($n = 3$ individuals for $A. unedo$ and $P. latifolia$ and $n = 1$ for $Q. ilex$) and to $A. unedo$ in 2000 ($n = 3$ individuals in the control plot and $n = 2$ in the drought-exposed one) (Table 3). Only trees in which sap flow data were available for $>80\%$ of days during the study were considered. Leaf-related sap flow and $G_0$ were averaged by species. Summer reductions of $Q_1$ were compared between species by fitting the relationship between $Q_1$ (%) (in relation to the species maximum reached at the beginning of the summer period) and SMD with the following function:

$$Q_1(\%) = 100e^{a_{SMD} - SMD_{o}} \times$$

(3)

where SMD is the soil moisture deficit at the beginning of the summer period, and $a$ and $b$, the fitted parameters, measure the sensitivity of $Q_1$ to SMD. When studying the relationship between $Q_1$ and SMD, summer days with mean daytime $D < 1$ kPa were removed from the analysis to avoid uninformative dispersion. Differences between curves were tested among species, treatments, or years by means of $F$-tests comparing the mean squares of the curvilinear regression with and without segregating data according to the variable under consideration (Potvin et al. 1990). When comparing species, periods or years, only individuals from the control plot were used, but whenever the treatment effect was clearly nonsignificant ($P > 0.15$), data were pooled by species. Comparisons were made only if there were at least two replicates within each level of the factor under consideration. Estimates of $G_0$ were regressed against $D$ by means of a logarithmic function (Schäfer et al. 2000, Oren and Pataki 2001). Although this procedure suffers from the lack of independence in the determination of the dependent variable, thus precluding statistical inferences, it is useful for quantifying the sensitivity of stomata to $D$ (Oren et al. 1999), particularly for comparative purposes. All statistical analyses were conducted with SPSS (Version 10.0.6, SPSS, Chicago, IL) and Statistica software (Version 5.95, Tulsa, OK).

**Results**

**Seasonal water relations and treatment effects**

The dynamics of soil water content ($\theta$) showed the typical pattern, with lowest values during the summer months of both years coinciding with periods with highest temperature and potential evapotranspiration (Figure 1). Water content was, on average, 9% units higher in the first 25 cm of soil than in the uppermost 40 cm (Figure 1c), probably because of higher porosity in the upper soil layers (J. Piñol, unpublished results). Considering all experimental plots, there was a significant reduction in $\theta_{0.25}$ associated with the drought treatment ($P = 0.03$; three-way, nested ANOVA), but no difference was found between the plots with sap flow sensors ($P = 0.28$). This was because the soil of the drought-treated plot was originally wetter than that of the control. However, the treatment was associated with a significant change in the difference in $\theta_{0.25}$ between the plots ($P < 0.01$; $t$-test comparing the difference before and after the onset of the treatment), causing the drought-exposed plot to be consistently drier than the control (Figure 2, maximum difference of about 15%).

There was no significant difference in water potentials between treatments for $P. latifolia$ or $Q. ilex$ ($P > 0.17$ for $\Psi_{PD}$ and $P > 0.13$ for $\Psi_{SMD}$), and thus the values were pooled by species in the subsequent analysis. In contrast, $A. unedo$ $\Psi_{PD}$ was significantly lower in the dry plot than in the control plot ($P = 0.02$) during spring–summer of 2000 (the only period in which this species was measured). No treatment effect was observed in $\Psi_{SMD}$ or in the difference between $\Psi_{SMD}$ and $\Psi_{PD}$ for $A. unedo$. Among species, $\Psi_{PD}$ was significantly lower in $Q. ilex$ than in $A. unedo$ ($P < 0.01$), and lower in $P. latifolia$ than in $Q. ilex$.
Predawn water potentials remained $> -1.2$ MPa, except during the summer months of both years when they reached minimum values ranging between $-2.7$ MPa ($A. unedo$ control) and $-6.2$ MPa ($P. latifolia$) (Figure 3a). Midday water potentials were also lowest in $P. latifolia$ ($P < 0.01$), but the difference between $A. unedo$ and $Q. ilex$ was not significant ($P = 0.09$) (Figure 3b). The difference between $\Psi_{MD}$ and $\Psi_{PD}$ decreased significantly during the summer in the three species ($P < 0.01$). The reduction was largest in $Q. ilex$ ($P < 0.05$), which reached < 0.1 MPa at the peak of the 2000 drought. The slope of the linear regression of $\Psi_{MD}$ against $\Psi_{PD}$ was lower for $Q. ilex$ (slope = 0.66; significantly $< 1$, $P < 0.05$) than for $P. latifolia$ (0.92; not different than 1, $P = 0.03$) (Figure 4). The regression between $\Psi_{MD}$ and $\Psi_{PD}$ was not significant for $A. unedo$ in the control plot. For $Q. ilex$ and $P. latifolia$, water potentials tended to be slightly lower in 2000 than in 1999 during the summer (Figure 3). This result is consistent with slightly lower soil water contents in 2000 (Figure 1), but should be interpreted with caution because few days were measured each year.

Sap flow and canopy stomatal conductance in relation to environmental variables

Leaf-related sap flow showed a similar temporal pattern for the three species studied in 1999 and for $A. unedo$ in 2000 (at both the control and the drought-exposed lots). In all cases, maximum values were reached during early summer (DOY = 168–185, depending on the species and year) (Figure 5) when $D$ started to increase and soil water content was still relatively high ($\theta_{0-25} > 0.23$ m$^3$ m$^{-3}$) (Figures 1 and 5). Maximum $Q_l$ was similar for the three species in 1999 ($P = 0.141$ when comparing $A. unedo$ and $P. latifolia$; $Q. ilex$ could not be compared statistically because of the lack of replicates) (Figure 5). In the case of $A. unedo$ and $P. latifolia$, this similarity was produced by similar sap flux densities and similar leaf-to-sapwood area ratios ($A_l:A_{SW}$) (Table 1). Instead, in the only $Q. ilex$ individual measured, consistently higher sap flux density (data not shown) was compensated by higher $A_l:A_{SW}$ (Table 1). In $A. unedo$, maximum $Q_l$ was 112% higher in 2000 than in 1999 ($P < 0.05$) (Figure 5).

During the summer, the continuous increase in SMD resulted in a reduction in $Q_l$ in all species (Figure 5). Vapor pressure deficit (and $E_T$) showed no clear pattern during the same period (Figure 5). The sensitivity of $Q_l$ to SMD was larger in $A. unedo$ than in $P. latifolia$ ($P < 0.05$), and much larger in the only measured $Q. ilex$ individual than in these other species (Figure 6a). Consistent with that result, estimated $k_{S-L}$ decreased sharply during the summer in the measured $Q. ilex$ individual, whereas it remained approximately constant in $P. latifolia$ ($P > 0.8$ when comparing early and late season val-
To investigate the causes of the drought-induced decrease in \( Q_i \), \( G_i \) was derived from \( Q_i \). The relationship between \( G_i \), and \( D \) in the three species changed during the study period (Figure 7), indicating increased stomatal closure as the drought progressed. The model corresponding to late summer (higher SMD) was divided by the early summer model for each species to calculate the percent reduction in \( G_i \), at a given \( D \) as a result of soil moisture depletion. In 1999, the reduction in \( G_i \) from early to late summer ranged from < 50% in \( P. \) latifolia to > 80% in the measured \( Q. \) ilex individual, with \( A. \) unedo showing an intermediate reduction of about 60% (Figure 8a). Late season reduction in \( G_i \) of \( A. \) unedo was larger in 2000 than in 1999, reaching > 85% at high \( D \) (Figure 8b).

The effects of the drought treatment were tested only in \( A. \) unedo, which was the only species that showed lower water potentials in response to the drought treatment in the year 2000. Despite similar maximum \( Q_i \) in the control and drought-exposed plots \((P > 0.4)\) (Figure 5), a treatment effect was apparent during the summer \((P < 0.05, \) Figure 6b), with higher sensitivity to increased SMD in the dry plot. At the end of the study period, \( Q_i \) in this plot was reduced to values about 50% of those in the control plot. Consistent with this pattern, the reduction in \( G_i \) from early to late summer was larger in the dry plot than in the control plot for any value of \( D \) (Figure 8b). In agreement with a treatment effect, \( k_{S-A} \) decreased significantly during summer 2000 in the drought-exposed plot \((P = 0.02)\) but not in the control plot \((P > 0.9)\) (Figure 3c).

Discussion

All studied trees showed similar patterns of seasonal water use, with maximum \( Q_i \) in early summer and acute reductions as the summer drought progressed (Figure 5). This pattern is typical in Mediterranean evergreen forests (e.g., Tognetti et al. 1998, Infante et al. 2001). Our estimates of daily sap flows are consistent with published values for trees of the same species in similar forests. Teixeira et al. (1998), for example, measured sap flow in \( Q. \) ilex and \( A. \) unedo in an evergreen forest in southern France. Although they provided daily sap flow estimates instead of \( Q_i \) or sap flux densities, their mean values from April to October \((3.7 \pm 0.4 \text{ kg day}^{-1} \text{ for } Q. \) ilex and \(2.1 \pm 0.4 \text{ kg day}^{-1} \text{ for } A. \) unedo\) are similar to ours \((2.7–4.1 \text{ kg day}^{-1} \text{ for } Q. \) ilex and \(0.8–1.2 \text{ kg day}^{-1} \text{ for } A. \) unedo\). The larger values for \( A. \) unedo are probably because their site was wetter than ours and their measured trees slightly larger.

Our results imply that the sharp reduction in \( Q_i \) in the studied trees as the drought progressed was caused by increased stomatal control over water loss. This is supported by the progressive reduction in estimated \( G_i \) during the summer (Figures 7 and 8) and by the low decoupling coefficients typical of \( Q. \) ilex woodlands (Infante et al. 1997) and suggested by our results (high ratio of boundary layer to stomatal conductance throughout the study period). Despite qualitatively similar patterns of seasonal water use, the magnitude of the decrease in sap flow during the summer and the seasonal dynamics of water potentials varied among species (Figures 3–6), suggesting that the responses to drought were also species-dependent.

The results agree with our initial hypothesis that the effects of drought on sap flow would be highest in \( Q. \) ilex and lowest in \( P. \) latifolia, and with previous leaf-based measurements showing that \( A. \) unedo and \( Q. \) ilex close stomata at relatively high water potentials (Castell et al. 1994, de Lillis and Mirgone 1994, Sala and Tenhunen 1994, Peñuelas et al. 1998). The smaller slope of the relationship between \( \Psi_{	ext{TR}} \) and \( \Psi_{	ext{MD}} \) for \( Q. \) ilex compared with that for \( P. \) latifolia (Figure 4) further supports this interpretation.

Data from the same study area show that \( A. \) unedo and \( Q. \) ilex are much more vulnerable to xylem embolism than \( P. \) latifolia (Table 1). Vulnerability to embolism and stomatal control are associated because plants must avoid dangerous losses of conductivity in the xylem caused by low water potentials (Oren et al. 1999, Sperry 2000; see below). We estimated native xylem embolism by combining the measurements of water potentials from this study with the vulnerability curves measured by Martínez-Vilalta et al. (2002b) in surface roots and stems of the three species studied and in the same area.

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**Figure 4.** Relationship between midday and predawn water potentials for \( A. \) unedo (Au), \( P. \) latifolia (Pl) and \( Q. \) ilex (Qi). Linear regression lines are depicted when significant, and the estimated regression slopes are shown in parentheses. The 1:1 relationship is also shown. Abbreviations: \( d \) = drought; \( c \) = control.
Predawn water potentials were assumed to provide an estimate of minimum xylem water potential in roots, whereas midday values were taken as a measure of xylem water potentials in stems. Estimates of xylem embolism increased sharply during the summer, reaching values >40% in all species and tissues at the peak of the drought (Figure 9). *Quercus ilex* experienced greater drought-induced xylem embolism than *P. latifolia* and, if the pattern observed in 2000 holds for 1999, *A. unedo* as well (Figure 9). Xylem embolism was lowest in *P. latifolia*, even though water potentials were much lower in this species than in *A. unedo* or in *Q. ilex*. In general, resistance to xylem embolism is well adjusted to the range of water potentials within which plants operate (Hacke and Sperry 2001). The higher % embolism in *Q. ilex* is in agreement with the larger decrease in $k_{SL}$ that we found in the *Q. ilex* individual that was measured during summer 1999 (Figure 3c).

Under steady-state conditions, and assuming a high degree of aerodynamic coupling with the atmosphere, stomatal conductance ($g_s$) is linked to $D$ by the following relationship (Oren et al. 1999):

$$g_s = K_L \Delta \Psi / D$$

where $K_L$ is leaf-specific hydraulic conductivity, and $\Delta \Psi$ is the gradient of water potential from soil to leaves. Because xylem embolism reduces $K_L$ at low water potentials and $\Delta \Psi$ tends to decrease with decreasing soil water potential (e.g., Figure 4), a reduction of $g_s$ (or $G_s$) at any given $D$ is expected as the soil dries (Figure 7). In agreement with our initial hypothesis, this reduction was greater in the only measured *Q. ilex* individual and smaller in *P. latifolia* (Figure 8), showing a high degree of correspondence with the values of native embolism estimated...
for each species (Figure 9). One advantage of this analysis is that differences among species in rooting extension and depth, which we have not studied, are partially incorporated via differences in $\Psi_{PD}$.

In A. unedo, the dynamics of $Q_l$ varied between years and as a result of the drought treatment (Figures 5 and 6). Higher maximum $Q_l$ and a sharper decrease during the summer in 2000 were probably associated with between-years differences in the distribution of precipitation (Figure 1). Cumulative precipitation to the beginning of the summer was 289 mm in 1999 and 393 mm in 2000, and what is probably more important, in 2000, high rainfall and soil humidity coincided with a period of high evaporative demand (Figure 1). This suggests that interannual variability in sap flow can be substantial in these communities (about a twofold change in maximum $Q_l$ between 1999 and 2000 in A. unedo) and should be incorpo-
rated when representative values of stand transpiration are required. High interannual variability in evapotranspiration has been observed in the same study area at the catchment level (Piñol et al. 1991).

Results for the treatment effect on *A. unedo* generally agreed with other through-fall manipulation experiments (Borghetti et al. 1998, Hanson et al. 2001 and references therein). The treatment caused a maximum decrease in the water content of surface soil of about 15%. This effect was associated with a 36% reduction in \( \Psi_{PD} \) (Figure 3), an increase in estimated \% loss of xylem conductivity (Figure 9), a significant decrease in \( k_{S,L} \) (Figure 3c) and a 50% reduction in \( Q_l \) at the peak of the 2000 drought (Figure 6b). Although the treatment was not truly replicated in this study, the similarity of the course followed by soil water content, leaf water potential, gas exchange and tree growth among study plots suggests that they were representative of the whole system. *Arbutus unedo* was the only species in which a treatment effect was observed in 2000; consistent with sap flow results, water exclusion decreased growth by 66% and modified the carbon isotopic composition of leaves (R. Ogaya and M. Mangirón, unpublished results).

In conclusion, our results suggest that the three species studied have contrasting strategies to deal with low water availability, as has been shown for other co-occurring Mediterranean species (e.g., Nardini et al. 1999). Although some of our conclusions must remain tentative because of small sample sizes, particularly in the case of *Q. ilex*, the following picture emerges. Low vulnerability to xylem embolism allows *P. latifolia* to maintain higher sap flow at lower water potentials than the other species. On the other hand, *A. unedo* and *Q. ilex* have greater stomatal control over water loss, thereby tending to avoid low water potentials that could cause a dangerous degree of xylem embolism ("runaway embolism," Tyree and Sperry 1988). Under extremely dry conditions, however, stomatal control in these species was insufficient to prevent extensive loss of hydraulic conductivity as a result of embolism, particularly in *Q. ilex*. The prediction that, in relation to water stress, *Q. ilex* at our study site is close to its distributional limit is in agreement with previous ecophysiological (Lo Gullo and Salleo 1993, Peñuelas et al. 1998) and modeling (Martínez-Vilalta et al. 2002a) studies, and with the acute impact that the 1994 drought had on *Q. ilex* populations (Lloret and Siscart 1995, Peñuelas et al. 2000). Given the increase in aridity predicted by climate change models in the Mediterranean basin (IPCC 2001) and already observed in NE Spain (Piñol et al. 1998), these results should be taken into consideration when predicting the impact of climate change on vegetation. However, acclimation to water stress or to increased atmospheric CO\(_2\) concentration could compensate, at
least in part, for a decrease in water availability (Gebre et al. 1998; Tognetti et al. 1998, Osborne et al. 2000).

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